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Influence of protective cultures on *Listeria monocytogenes* in fermented sausages: a review

Einfluss von Schutzkulturen auf Listeria monocytogenes in Rohwürsten

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Summary

Within the concept of protective technologies in the production of fermented sausages, in addition to known hurdles such as nitrites, NaCl, low water activity and low pH, of great importance is also the biopreservation procedure. This procedure involves the use of lactic acid bacteria, their metabolites and protective substances for the purpose of achieving antagonistic effects on the undesirable microflora. The importance of biopreservation is optimally manifested in the bacteriostatic and/or bactericidal action of protective cultures and bacteriocins on *Listeria monocytogenes* in various foods, including fermented sausages. Biopreservation is an additional factor in the production of safer food, but it alone can not warrant microbiological safety. In order to have purport and effect, this method must be a superstructure to good manufacturing and good hygienic practice.

Keywords: protective cultures, bacteriocins, *Listeria monocytogenes*, fermented sausages

Zusammenfassung

Im Rahmen der Haltbarmachung bei der Produktion von Rohwürsten hat neben den bekannten Faktoren wie Nitrit, Salz, niedrige Wasseraktivität und geringer pH-Wert der Prozess der Biokonservierung Bedeutung. Dies umfasst den Einsatz von Milchsäurebakterien sowie von deren Stoffwechselprodukten und Schutzfaktoren mit dem Ziel einer antagonistischen Wirkung auf unerwünschte Mikroflora. Die Bedeutung der Biokonservierung lässt sich optimal beobachten an der bakteriostatischen und/oder bakteriziden Wirkung von Schutzkulturen auf *Listeria monocytogenes* in verschiedenen Produkten, vor allem in Rohwürsten. Die Biokonservierung ist ein zusätzlicher Faktor in der Produktion von sicheren Nahrungsmitteln, jedoch kann sie die mikrobielle Unbedenklichkeit nicht alleine garantieren. Um eine Wirkung zu zeigen, muß diese Methode in Verbindung mit einer guten Herstellungspraxis sowie einer guten Hygienepraxis eingesetzt werden.

Schlüsselwörter: Schutzkulturen, Bakteriozine, *Listeria monocytogenes*, Rohwürste

Introduction

Fermentation is one of the oldest processes of meat preservation, which depends on the biological activity of lactic acid bacteria (LAB) i. e. on the production of different metabolites capable of suppressing the growth of undesirable microbial flora (Ross et al., 2002; Hutkins, 2006). Knowledge about the role of microorganisms in the food fermentation process dates long ago, so that individual strains, considered fit from the technological and hygienic point of view, have been introduced in the production of fermented foodstuffs in form of starter cultures. In case of fermented sausages, these are lactic acid bacteria, prevailingly species of the genus *Lactobacillus* and *Pediococcus* (Hutkins, 2006). Antimicrobial activity of LAB is manifested by inhibition of other microorganisms through competition for nutritive ingredients and/or production of one or more antimicrobial metabolites such as organic acids (lactic, acetic), hydrogen peroxide, antimicrobial enzymes, bacteriocins and reuterin (Holzapfel et al., 1995). The use of bacteriocin-synthesising strains as protective cultures and the direct use of the indicated, so-called natural protective substances (bio-preservatives, bacteriocins) in the production of fermented foods are components of the protective concept in food industry (Montville and Matthews, 2005).

Biopreservation

Ancient methods of food preservation, such as salting, fermentation, smoking, drying etc., have not been abandoned, but are still employed in the production of meat products. The improvement of industrial processing methods has resulted in the development of different additional protective methods, which have the same aim as in the early days of food preservation, i. e. prevention of spoilage, inhibition of growth of spoilage microorganisms and pathogens or prolongation of the product's shelf life (Zdolec, 2007). In this sense, various chemical substances in the form of additives have been used, as well as protective microbial cultures, different physical procedures (irradiation, thermodynamic processes and pulsed electric field) and packing under modified atmosphere (Devlieghere et al., 2004.).

Hurdle technology, as a specific concept, includes a combination of the existing (temperature, water activity, pH, Eh, preservatives etc.) and novel techniques of preservation (packing under gas, treatment with high pressure, biopreservation), the aim of which was to establish selective protective factors in which pathogenic and spoilage microorganisms could not survive (Leistner and Gorris, 1995). The idea of biopreservation comprehends the process of fermentation and preservation as a method designed for prolongation of shelf life and enhancement of food safety by the use of microorganisms and/or their metabolites (Ross et al., 2002). As regards meat and meat products, biopreservation includes the addition of bacteriocinogenic LAB cultures, non-purified bacteriocins, fermentation culture or bacteriocinogenic culture concentrate, purified or semi-purified bacteriocin, as well as the addition of mesophilic LAB as a form of protection in case of temperature abuse (Hugas, 1998). Lücke (2000) has pointed out that the production of lactic acid is the main mechanism of action of protective cultures on the competitive microflora, while the effect of bacteriocins is reduced by their inactivation in meat and by the development of resistance. Also, the use of bacteriocinogenic lactic acid bacteria can not yield satisfactory results in the prevention of intestinal infections induced by gram-negative bacteria (e. g. EHEC) and in the prolongation of shelf life of meat kept under aerobic conditions.

Several factors should be taken into consideration when selecting bacteriocinogenic lactic acid bacteria for use in a certain food product. These are GRAS status (Generally Recognised As Safe), spectrum of inhibition of microorganisms, thermal stability of bacteriocins, risk for human health, improvement of safety and quality and a highly specific activity (Holzapfel et al., 1995). Modern research works in molecular biology are directed towards the development of a new generation of protective cultures. Future researches of bacteriocinogenic cultures will be aimed at:

- better and target selection and screening methods,
- genetic optimisation with recombinant DNA technology,
- broadening of the spectrum of activity in combination with additives from food or natural ingredients or with high-pressure technology or pulsating electrical field technology (Devlieghere et al., 2004).

Various authors have also pointed out that in the process of biopreservation attention should be paid to the cultures of lactic acid bacteria not producing bacteriocins. Their antagonism is based on the production of lactic acid and acidification, production of other antimicrobial substances and competition for nutritive ingredients in a substrate.

Protective cultures vs. *L. monocytogenes* in fermented sausages

Human listeriosis is an alimentary infection caused by bacteria of the species *Listeria* (*L.*) *monocytogenes*. The

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causal agent shows peculiar characteristics such as high resistance in, for many bacteria, pernicious conditions. For example, it survives freezing, multiplies at low temperatures during the cooling process and survives in acid and alkaline media, at low water activity and increased salt concentration. Consequently, its survival and multiplication are possible in various types of foods and under different storage conditions. *L. monocytogenes* is an omnipresent organism in the environment, and has been isolated from many types of food and production facilities (Loncarevic, 1998; Kozačinski and Hadžiosmanović, 2001; Thévenot et al., 2005a). According to the report of de Valk et al. (2005), the number of food-related cases of listeriosis in humans in Europe is not negligible.

Survival and growth of *L. monocytogenes* in fermented sausages largely depend on the sausage type, conditions during fermentation (micro- and macroclimate), starter cultures used and adaptability of the pathogen to meat substrate (Encinas et al., 1999; Thévenot et al., 2005a, 2005b; Zdolec et al., 2005). Within the concept of protective technologies in the production of fermented sausage, the use of protective bacteriocinogenic cultures has shown to be an additional protective factor against *L. monocytogenes* (Tab. 1).

Recently performed studies within the EU project "Safety of traditional fermented sausages: research on protective cultures and bacteriocins", have been focused on the antilisterial capacity of bacteriocin-producing cultures of *Lactobacillus (Lb.) sakei* I151, I154 and I155 in various Central-European and Mediterranean traditionally fermented sausages – Hungarian salami, Bosnian sucuk, "Sremska" sausage (Serbia) and Croatian sausage (Hadžiosmanović et al., 2005; Čaklovica et al., 2006; Gasparik-Reichardt et al., 2006; Drosinos et al., 2006; Zdolec et al., 2007). Use of *Lb. sakei* cultures in the Hungarian salami resulted in an about 2 $\log_{10}$ lower pathogen count during the 28 day interval of ripening (from the initial 5.5 to 3.5 log cfu_{10} g^{-1}), while in the control sausage a reduction by about 1.5 $\log_{10}$ was observed. A complete elimination of *L. monocytogenes* from the stuffing of the Bosnian sucuk was achieved during the ripening period (28 days) with the use of protective *Lb. sakei* strains (reduction about 5 $\log_{10}$). This, however, was not the case with the control sausage (reduction from the initial 4.85 to 3.83 log cfu_{10} g^{-1}). Similar results have been recorded in case of the "sremska" sausage. *L. monocytogenes* was not found in the finished product (after 28 days) owing to the use of protective strains (reduction 4.0–4.5 $\log_{10}$), but it survived in the control sausage (< 2 log cfu_{10} g^{-1}). In our study, the *L. monocytogenes* population was reduced below the detection limit in sausages with *Lb. sakei* I155 already on day 14, while in control samples and sausages with *Lb. sakei* I151 and I154 by the end of ripening (28th day) (Fig. 1). Study results suggest the contribution of bacteriocinogenic cultures of *Lb. sakei* to the safety of fermented sausages due to an evidently enhanced reduction of the inoculated *L. monocytogenes* population.

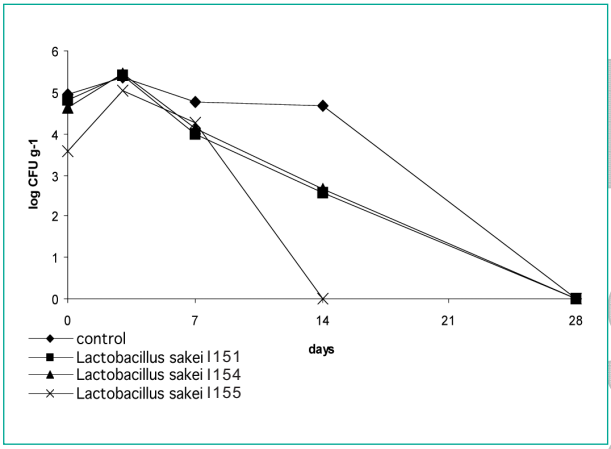

FIGURE 1: *Changes in L. monocytogenes count* during the ripening of Croatian fermented sausages inoculated with protective cultures of Lb. sakei.*

*mean of three fermentations.

TABLE 1: *Examples of implementation of bacteriocinogenic LAB cultures and bacteriocins in fermented sausages*

Culture/bacteriocin	*Purpose*	Effect	Reference
Pediococcus acidilactici	Use in control of *L. monocytogenes* in dry fermented sausages	Reduction of pathogens during fermentation and drying due to pediocin activity in situ	Foegeding et al. (1992)
Lactobacillus sakei and *Lactobacillus curvatus*	Implementation in two different types of sausages inoculated with *L. monocytogenes*	Reduction degree of *Listeria* depends on production technology and strain adaptation	Hugas et al. (1997)
Pediococcus acidilactici PA-2 and *Lactobacillus bavaricus* DF126	Application in fermented sausages inoculated with different levels of *L. monocytogenes*	Level of reduction is correlated to initial count of *Listeria*. LAB cultures enhanced the decrease of pathogen.	Lahti et al. (2001)
Lactobacillus plantarum 423 and *Lactobacillus curvatus* DF126	Control of *L. monocytogenes* in ostrich fermented sausages	2–3 $\log_{10}$ reduction of pathogen within 9 days, followed by increase to initial count due to loss of bacteriocin activity or resistance	Dicks et al. (2004)
Lactococcus lactis subsp. *lactis* M	Use in fermented sausages «marguez» with nitrites and *L. monocytogenes*	Enhanced reduction of pathogens with presence of culture. Nitrites reduced antilisterial activity of bacteriocin.	Benkerroum et al. (2003)
Lactococcus lactis subsp. *lactis* LMG21206 and *Lactobacillus curvatus* LBPE	Production of fermented sausages inoculated with *L. monocytogenes* (2–3 $\log_{10}$ CFU/g)	Elimination of *Listeria* in earlier phase compared with control or sausages with non-bacteriocinogenic culture	Benkerroum et al. (2005)
Enterocin CCM 4231	Use in production of Hornad salami inoculated with *L. monocytogenes*	3 $\log_{10}$ lower count of pathogen in final product than in control (4.7 $\log_{10}$ CFU/g)	Laukova et al. (1999)
Enterocin AS-48	Production of sausages inoculated with *L. monocytogenes* and different concentrations of bacteriocin	Reduction degree of *Listeria* is correlated with enterocin concentration	Ananou et al. (2005)

Conclusions

Microbiological quality is an important factor of food safety, which can be influenced by a large number of food processing procedures. It is well known that optimum conditions are a prerequisite for the growth and multiplication of microorganisms (of any species). Thus, the production technologies employed in the production of meat products have a direct impact also on the stimulation of growth of target microbial species, whilst others can be inhibited or destroyed with the same methods or a combination of methods.

The microbiological stability of dry fermented sausages is based on physicochemical changes during ripening, mainly lowering the pH, increasing salt content and decreasing water activity. Besides that, the microbiological interactions in the filling are of great importance, too, with particular regard to antimicrobial mechanisms of lactic acid bacteria. Effectiveness of LAB bacteriocins in reduction of potential microbial hazards in fermented sausages such as *L. monocytogenes* is associated with several factors such as number of bacteriocin-producing strains, their adaptability to meat substrate, number and origin of target organisms, sausage composition, conditions during ripening (temperature, pH, moisture) and bacteriocin stability. During the storage of fermented sausages and other foodstuffs the antimicrobial capacity of bacteriocins could be enhanced due to combination with other methods in particular packaging under modified atmosphere or vacuum. Biopreservation by itself can not warrant microbiological quality of food products, and in order to have purport and effect, this method must be a superstructure to good manufacturing and good hygienic practice.

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